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Neural Stem Cells Are Blasting Off

Minireview

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Introduction

Over the past 5 years, there has been a flurry of excitement over the identification of putative neural stem cells. Why all the fuss? Stem cells, as the name implies, are cells from which other cell types arise and thus are of great interest to developmental biologists. The most general type of stem cell is the fertilized egg; from this single cell arise the myriad cell types that constitute a mature multicellular animal. Operationally, however, the appellation is given to cells with far less developmental potential; in a given tissue, one can speak of stem cells that have the ability to give rise to all of the differentiated cell types associated with that specific tissue. Several properties of these cells provide a useful operational definition. Specifically, stem cells are undifferentiated cells capable of proliferation, self-renewal, and "asymmetric" division. The latter allows for self-renewal (i.e. the production of new stem cells) as well as the production of differentiated progeny. All tissues contain tissue-specific stem cells during organogenesis, as do adult tissues capable of regeneration. In such tissues, latent stem cell populations are sparked into action by stress, injury, etc. (reviewed by Potten and Loeffler, 1990).

What then is a neural stem cell? In a strict sense, it should be a cell that gives rise to a variety of neurons and glia. Identification of such cells in the embryonic nervous system is a recent feat, but given the seeming lack of neural regeneration in the adult mammalian central nervous system (CNS), the idea that latent stem cells exist here, too, has seemed heretical. While this view is now in flux, current discussions of neural stem cells are characterized by ambiguous vocabulary and a plethora of disparate observations. A resolution of this muddle is not merely of academic interest. An understanding of neural stem cell biology will have profound consequences for the treatment of neurological diseases through ex vivo manipulation of such cells for transplantation or in vivo activation of quiescent neural stem cells to promote healing from within. In this minireview, we will address several issues. First, what is the evidence for the existence of neural stem cells in mammals? Second, how do regional differences in the nervous system affect neural stem cell behavior? Finally, what are the similarities among reported neural stem cells?

Central to our definition of stem cells is the property of self-renewal. The most direct method to assess the self-renewal of putative stem cells is to mitotically expand candidate cells in isolation. By subcloning the resultant progeny, one can examine the founder cell's ability to produce differentiated cell types and additional stem cells. Cells capable of proliferation and neurogenesis in such a paradigm, but which fail to self-renew, are best termed progenitor cells rather than stem cells. Detailed analyses demonstrating self-renewal have been accomplished only within a few neural structures: the neural crest, the embryonic cerebral cortex, and the embryonic striatum.

Peripheral Neural Stem Cells

Clonal primary cultures of rat neural crest have revealed that single cells that coexpress nestin and low affinity nerve growth factor receptor can produce large colonies containing neurons, Schwann cells, and smooth muscle cells (Stemple and Anderson, 1992; Shah et al., 1996). Subcloning experiments showed that these cells self-renew, and hence these cells were designated neural crest stem cells (NCSCs). The fact that these cells can be grown in clonal culture has allowed the direct assessment of the activity of purified growth factors. In recent experiments, NCSCs were found to differentiate in response to several different growth factors along different pathways (Shah et al., 1994; 1996). In response to neuregulin, nearly 80% of NCSCs respond to form only Schwann cells. By contrast, neurogenesis is induced by bone morphogenic proteins 2 and 4 (BMP2/4), and smooth muscle cells are generated in response to transforming growth factor β1. It appears that these growth factors act directly to convert NCSC into differentiated progeny. This is most apparent with BMP2/4 treatment. Within 6 hr of such treatment, well before overt neuronal differentiation, NCSCs express MASH1, a transcription factor that is critical for the differentiation of certain neuron subtypes. Furthermore, this treatment results in the complete loss of stem cells, i.e., treated cells lose their capacity to self-renew. Hence, the ability of NCSCs to self-renew can be overridden by environmental signals, and this may explain the paucity of such cells in the adult.

It is interesting to note that there is a site of peripheral neurogenesis that is likely to host stem cells throughout adulthood, the olfactory epithelium (OE). The OE is a site in which neurogenesis occurs throughout the lifetime of all vertebrates. While it is possible to culture OE-derived cells and demonstrate in vitro neurogenesis (Calof and Chikaraishi, 1989; Pixley, 1992; Mahanthappa and Schwarting, 1993), it has been difficult to identify a cell that qualifies as the olfactory stem cell. It is thought that the putative stem cell resides in the basal OE, but identification of such a cell is a topic of current research. Nevertheless, it is noteworthy that mice in which expression of the MASH1 gene has been deleted, only show severe deficits in numbers of sympathetic ganglion neurons (an NCSC derivative) and OE neurons (Guillemot et al., 1993). Thus, neural stem cells in the periphery may share common properties.

Central Neural Stem Cells

In the cerebral cortex, self-renewal has been directly demonstrated for a multipotent stem cell isolated from embryonic rat. Using medium conditioned by astrocytes and meningeal cells, Davis and Temple found that about

7% of clones produced hundreds of cells and divided for weeks (1994). About 40% of these large clones produced neurons, oligodendrocytes, and astrocytes. Self-renewal of the stem cell was demonstrated by the subcloning of several clones. For each of these clones, at least one subclone was of the large, multipotent type. This system should allow a detailed examination of the relationship between cortical stem cells and their differentiated progeny, and the identification of factors controlling differentiation decisions. In particular, this system will be useful to establish the relationship between neuronal and glial lineages.

Under different culture conditions, Reynolds and Weiss have identified a CNS stem cell from the embryonic striatum. In low density culture of embryonic striatum, a subset of cells were found to proliferate when grown in suspension in the presence of epidermal growth factor (EGF) (Reynolds et al., 1992). These cells are reported to form clonal spheres of nestin expressing cells, few of which express markers of differentiation. When the spheres are dissociated and grown on an adherent substrate in the absence of EGF, they differentiate into mixed colonies of neurons, astrocytes and oligodendrocytes. Additional studies report that these spheres can be dissociated to generate clonal secondary spheres that retain the ability to produce neurons, astrocytes and oligodendrocytes (Reynolds and Weiss, 1996).

The subependymal zone adjacent to the striatum is found to be a source of an adult version of the EGF responsive multipotential CNS stem cell (Reynolds and Weiss, 1992; Morshead et al., 1994). As with the embryonic form, the EGF-responsive adult stem cell expresses nestin and undergoes extensive proliferation in suspension cultures to form large spheres that differentiate when plated on an adherent substrate. In a similar study, basic fibroblast growth factor (bFGF) was also found to stimulate sphere formation in cultures of adult striatal subependyma (Gritti et al., 1996). Importantly, in the latter study, a clonal analysis of bFGF-stimulated spheres demonstrated that these cells can self-renew and generate neurons, oligodendrocytes, and astrocytes.

It is difficult to know whether NCSCs, cortical stem cells, and striatal stem cells are distinct cell types, given the fact that they have been identified using such different culture systems. Identity among these cells seems unlikely; for example, NCSCs produce neurons in response to BMPs 2 and 4, while embryonic striatal stem cells produce astrocytes in response to a variety of BMPs including BMP2 and 4 (Gross et al., 1996). Nonetheless, it would be informative to compare the three cell types directly under identical culture conditions.

Progenitors

A number of reports detail the properties of a variety of neural progenitors. One of the most extensively studied neural progenitors, oligodendrocyte-type II astrocyte (O-2A) progenitor, is the most likely source of oligodendrocytes throughout the CNS. Originally isolated from embryonic rat optic nerve, the O-2A progenitor produces both oligodendrocytes, marked in culture by morphology and the expression of galactocerebroside (Gal-C), and type II astrocytes marked by expression of the intermediate filament, glial fibrilary acidic protein (GFAP) (reviewed by Barres and Raff, 1994; Raff, 1989). The choice of cell fate was found to be controlled by a number of growth factors. For example, the combination of platelet derived growth factor (PDGF) and bFGF allows the extended proliferation of undifferentiated progenitors. Ciliary neurotrophic factor (CNTF) was found to induce type II astrocyte differentiation in the presence of extracellular matrix. Pro-oligodendrocytes, expressing O4 glycoside but not Gal-C, are generated in response to PDGF. Recently, neuregulin has also been shown to act as a mitogen and to prevent differentiation of the O-2A progenitor and its derivatives (Canoll et al., 1996). Given these observations, the O-2A progenitor may well be a stem cell, albeit of more restricted differentiation potential than the above-described stem cells. Nevertheless, such a designation will require formal clonal analysis that includes subcloning to demonstrate self-renewal. Further, the significance of the type II astrocyte has been called into question since attempts to identify an in vivo counterpart have failed (Williams et al., 1991), and histological and cell culture evidence support the view that astrocytes can derive from radial glia (Culican et al., 1990). Thus the O-2A counterpart in vivo may generate solely oligodendrocytes.

The study of Kilpatrick and Bartlett (1993) demonstrated that large multipotent clones can be obtained from embryonic mouse telencephalon/mesencephalon cells when grown in the presence of serum and bFGF on an adherent substrate. Although this study did not directly demonstrate self-renewal, $>$40% of the clones contained 3000–4000 cells by 10 days in culture. One quarter of all clones contained neurons and more than half of the neuron-containing clones also contained astrocytes. Interestingly, mature oligodendrocytes were never found to develop in these cultures.

Regional Identity and Stem Cell Behavior

Stem cells provide one mechanism for the generation of cellular diversity. For example, within a given region of the brain, a stem cell can generate a variety of cell types. In addition, neurons from different regions of the brain can have distinct region-specific properties. One major question in stem cell biology concerns the developmental potential of a stem cell. Several studies directly address the issue of developmental potential of isolated neural progenitors through heterotopic transplantation.

Recently, insightful experiments have been performed using adult hippocampal progenitors (AHPs). Cells isolated from adult hippocampus were found to proliferate in response to bFGF in a chemically defined medium. Under these culture conditions, the hippocampal cells were found to survive, and varying percentages express neuronal or glial markers. Strikingly, it is reported that these cells can be maintained in culture through multiple passages for 1 year, and when transplanted integrate and generate mature granule cell neurons in host hippocampus without forming tumors (Gage et al., 1995).

Depending on the site of transplantation, AHPs demonstrate unforeseen plasticity (Suhonen et al., 1996). When these cells were grafted into the rostral migratory pathway, the normal source of olfactory bulb (OB) granule cells, they generated mature OB neurons. Some of the grafted neurons expressed tyrosine hydroxylase

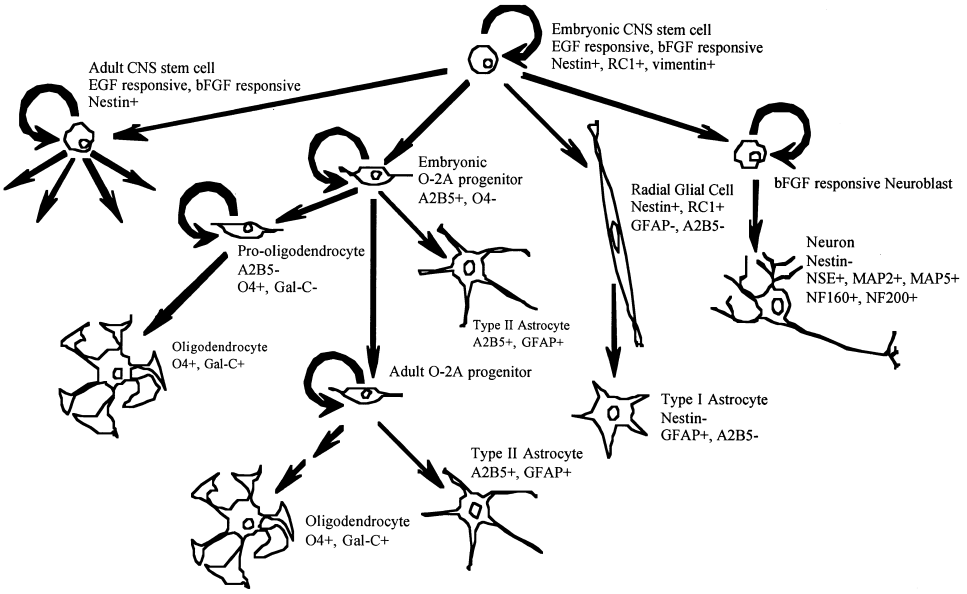

Figure 1. Idealized Lineal Relationships between the Major CNS Cell Types

In an effort to unify the studies of neural stem cells, we offer this diagram depicting the relationships between the cell types discussed. At the top is the embryonic CNS stem cell, which can self-renew and produce differentiated progeny, perhaps by a gradual restriction mechanism where intermediate cell types such as the O-2A progenitor or neuroblasts serve as the immediate precursors of the terminally differentiated cell types.

(TH), a neurotransmitter biosynthetic enzyme not normally expressed by hippocampal neurons. When the same cells were transplanted to the cerebellum, however, they failed to generate neurons though they survived at rates comparable to hippocampus or RMP grafts. Interestingly, $GFAP^+$ cells were produced by the AHPs in all three sites.

Self-renewal has not been formally demonstrated in clones of AHPs nor has the relationship between AHP-derived neurons and glia been established. It would be interesting to know if single AHP cells can form neurons and glia. Alternatively, AHP cells may be of two types that proliferate in bFGF–one that is essentially a neuroblast capable only of generating neurons, and one that is a glioblast that proliferates simultaneously with the neuroblasts. Given the fact that AHPs and adult striatal progenitors show a similar response to bFGF (Gritti et al., 1996), these cells may represent essentially the same stem cell subject to slightly different culture conditions. A direct comparison of clones derived from the two populations under identical culture conditions would clarify the relationship between the two adult progenitor cell types.

While AHP cells failed to adopt cerebellar neuron identities, progenitors of cerebellar origin can adopt hippocampal identities. The external germinal layer (EGL), which normally serves as the source of cerebellar granule cell progenitors, is an actively proliferating region through the first few weeks of postnatal life in rodents. Labeled cells taken from newborn rats or transgenic mice have been transplanted into the dentate gyrus of newborn rats. When host animals were analyzed, hippocampal granule cell neurons were found to develop from transplanted progenitors. Importantly, transplant-derived hippocampal neurons were found to express calbindin and to express c-fos in response to kainic acid treatment. Normal cerebellar granule cells do not express calbindin or display the kainic acid response. Hence, metencephalic progenitors were found capable of adopting telencephalic fates when subjected to the environment of the dentate gyrus (Vicario-Abejon et al., 1995).

Can these two studies be reconciled? It is important to note that the EGL only exists for a few weeks after birth. In the study of Suhonen et al. (1996), AHPs were grafted to the adult (>3 month old) cerebellum, after granule cell neurogenesis had taken place. Therefore, it may not be particularly surprising that the cues necessary to signal cerebellar differentiation are no longer present. Thus, it would be informative to graft AHPs into the newborn cerebellum to examine whether AHP plasticity is contingent on the persistence of appropriate environmental cues. Interestingly, immortalized EGL cell lines transplanted into developing cerebellum give rise to granule and basket cell neurons (Snyder et al., 1992), and when transplanted into the lateral ventricles of newborn mice, display an ability to populate regions of the brain as diverse as the cerebral cortex (Snyder et al., 1995). Immortalization may somewhat alter the regional specificity of the cells since untransformed EGL progenitors apparently produce only granule cell neurons. Nevertheless, it is possible that some progenitor cells of cerebellar origin show greater developmental potential than those of the hippocampus.

Unifying Principles?

Here, we have summarized just a fraction of the many recent studies concerning mammalian neural progenitors. Taken together, these studies provide convincing evidence for at least one type of neural stem cell for the CNS and PNS, respectively. There may be, however,

several different CNS and PNS stem cell types. Differences in cell culture conditions, regional sources of material, and varying differentiation assays make it difficult to compare studies and draw them into a consistent framework. Nevertheless, the similarities that exist suggest a common relationship between an archetypal CNS stem cell and its progeny (Figure 1). The data suggest that stem cells derived from different regions of the CNS display a similar growth factor responsiveness. Furthermore, regional differences in the brain appear to control the specific type of neurons that are formed.

Are there relationships among the various cells described above? All of the known multipotential neural stem cells, capable of forming both neurons and glia, express nestin. Nestin is subsequently expressed in glial derivatives of the NCSC and of CNS stem cells. Specifically, nestin is expressed in nonmyelinating Schwann cells, radial glia, and activated astrocytes. It is thus interesting to consider that these nestin-expressing glial derivatives may retain multipotential stem cell activities. It is also worth noting that stem cells may beget self-renewing cells of progressively limited developmental potential. In this light, cells such as the O-2A progenitor may truly be stem cell intermediates through which the cortical and striatal stem cells generate both astrocytes and oligodendrocytes. Similarly, a direct comparison of stem cells derived from embryos and those derived from adults will be important to examine the extent to which self-renewing stem cells from the latter retain the developmental potential of the former. Thus, relationships are bound to exist, but current studies only begin to address these issues.

How might one understand the origins of neural diversity in the developing vertebrate and its maintenance in the adult? To answer this question, it is important to realize that there are two conceptually separable issues: regional specification and stem cell differentiation. While progress is being made toward the molecular and cellular bases of both phenomena, the two have yet to be effectively integrated. It seems most fruitful to view the stem cell populations that constitute the primordial neuroepithelial sheet as the substrate on which patterning mechanisms act. Regional fate cannot be adopted in the absence of a multipotential stem cell. Likewise, for a stem cell to produce a particular neural or glial type, the appropriate positional signals (intrinsic and extrinsic) must be present. Hence, the problem becomes one of understanding how regional specification mechanisms interact with stem cell mechanisms to control differentiation.

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